Guideline for Completing the NIH Intramural Animal Study Proposal Form

The Animal Welfare Act (AWA) Regulations and the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy) require review and approval of an Animal Study Proposal (ASP) by the Institute/Center (IC) Animal Care and Use Committee (ACUC) prior to the initiation of any research activity involving vertebrate animals. All regulatory and guidance documents referred to in these instructions are provided on the Office of Animal Care and Use website under the ARAC Guidelines, NIH Policies, or Regulations & Standards subpages.

GENERAL INSTRUCTIONS:

These instructions are intended to aid any investigator in the completion of the ASP form but are targeted to new investigators. The goal of this guideline is to help an investigator prepare an ASP that can be approved by the IC ACUC in a single round of review. The most common mistake made by investigators that leads to delay of ASP approval is the inclusion of more detail in the ASP than is required by the regulations or requested on the form. The excess detail invites questions from ACUC members which require an investigator's time to clarify and additional ACUC time to review and approve. To avoid this problem, the best advice is to seek the guidance of a senior investigator who is familiar with animal research and the ACUC, an ACUC member and/or your IC Animal Program Director (APD) for help during the preparation of the ASP. There are many opportunities for "pre-review" during the protocol development stages that ensure that the ASP submitted to the ACUC is in near "final form". A well-constructed ASP will facilitate the review process so that your animal research can proceed rapidly. If you are able to attend the ACUC meeting to directly answer questions from ACUC members about your ASP, this too will speed up the approval of the protocol.

Do not underestimate the need to contact your IC ACUC Safety representative (301-496-2960) during the protocol development stage. Each IC has an NIH Division of Occupational Health and Safety (DOHS) Safety and Health Specialist assigned to work with their ACUC, contact your IC ACUC Coordinator to identify your Safety and Health Specialist. Many ASPs are not approved expeditiously because the safety information is incomplete. Likewise, you should contact the veterinarian in the facility where your animals will be housed to be sure that the facility can support the animal procedures proposed (your IC ACUC Coordinator can provide this individual's name). These two critical contacts must be made before the protocol is submitted for review to avoid unnecessary delays in implementing the research.

The information that you provide in the ASP should represent an outline of the research to be conducted that involves animals. As outlined in the US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training (US Government Principles), the ASP will be evaluated to assure that the research proposed is an appropriate use of the animals, and that technical assistance and appropriate facilities are available to support the research. Once the ASP is approved, however, portions of the ASP that are not exempt from disclosure under the Freedom of Information Act (FOIA) are subject to release if a request is made under the FOIA. NIH would withhold any confidential information contained in the ASP that is exempt from disclosure under the FOIA in consultation with your IC FOIA office.

SECTION A: ADMINISTRATIVE DATA

Principal Investigator: There may only be one Principal Investigator (PI) on an ASP. The PI assumes all responsibility for the animals, the ASP and its execution. Other investigators should be listed in "Key Personnel".

Bldg/Room, E-mail, Telephone, FAX: Provide complete and accurate information, as this will be utilized for the life of the ASP.

Submitted ASP: Indicate whether this is an initial submission, renewal (every three years) or modification (such as a change in PI or other significant change) of an existing ASP. If this is not an initial submission, enter in the number of the approved proposal or renewal ASP number (if available).

Key Personnel: List all personnel who will be conducting procedures involving animals under this ASP. All personnel listed in this section will need to undergo NIH, IC, and facility training and be enrolled in the Animal Exposure Program (AEP). Investigators not handling animals or not conducting procedures on live animals (for example, investigators who make use of rodent tissues collected by someone listed on the ASP) need not be listed here. **NOTE:** Investigators handling *tissue sor body fluids* from 'Old-World' NHP species (i.e., Rhesus, Pigtails, and Cynomolgus Macaques, Baboons, African Green Monkeys, Gibbons, etc.) outside of an *ACUC-approved NHP animal research facility*, are required to register their work and experimental use of these materials with the NIH Institutional Biosafety Committee (IBC) via a Pathogen Registration Document (PRD). Further details are provided in PM 3044-2 Protection of NIH Personnel Who Work With Nonhuman Primates.

SECTION B: ANIMAL REQUIREMENTS

Species, Age/Weight/Size, Sex, Stock or Strain: Provide specific information regarding these characteristics of the animals. List all animal vendors, stocks, and strains that will be used. If you are using a transgenic or knock-out strain of animal, provide the background strain(s) and the designation of the genetic mutation. Consult the nomenclature rules (http://www.informatics.jax.org/mgihome/nomen/index.shtml) and your IC APD, as proper nomenclature will be essential for ordering and tracking animals.

Source: Rodents are generally ordered from approved vendors through the **Division of Veterinary Resources** (DVR) Centralized Animal Procurement System (CAPS). For all aspects of the animal procurement process, contact your IC APD. If rodents or rodent products are obtained from a source other than the DVR list of "approved sources" a "rodent import application" form must be filled out and submitted to your IC APD and ultimately approved before the animals or products are brought to NIH.

Holding Location(s): Indicate where animals are proposed to be housed (building and room). **NOTE:** If it is necessary to hold animals anywhere outside of a core animal facility for more than 12 hours this should be indicated in the ASP. In addition, if you need to hold the animals in this space for more than 24 hours, a "satellite" facility must be established and approved by your ACUC. Contact your APD if you think your animals will need housing outside of a designated animal facility.

Animal Procedure Location(s): Provide the building and room number for all laboratory or special animal activity spaces, or animal facility spaces that will be used for all live animal procedures to include surgeries, euthanasia, and tissue harvesting.

Number of Animals: This section should reflect the total number of animals estimated and justified under Section E3 for each of the three years of the ASP (if necessary). It is recommended to estimate the number of animals in Section E3 prior to filling in numbers in Section B. If multiple species are used, provide the numbers separately for each species. If the study is a renewal involving USDA-regulated species, remember to account for animals to be carried over from the previous protocol.

SECTION C: TRANSPORTATION

Animals may need to be transported to different animal procedure or holding facilities (e.g. the Mouse Imaging Facility). If you are not proposing animal transfer, write "none" here. For ASPs involving animal transfers, include the destination, cite the ARAC "NIH Animal Transportation Guidelines" and consult with your APD about routes and the type of cages required. To assure the security and safety of the animals and humans involved, requirements for proper transportation of animals are described in the ARAC Guidelines for NIH Animal Transportation for rodents and non-rodents. In addition, transport of animals through the Clinical Center must comply with the "Research Animal Transport for the NIH Clinical Center".

SECTION D: STUDY OBJECTIVES

This section is to describe the objectives of the work in language lay people can understand. Excessive detail here can be distracting to the members of the ACUC, especially the lay members, requiring clarifications which lead to delays in the approval of the ASP. Provide an approximately 300-word summary of the research objectives. The summary should explain why the work is important and how the results of the study might benefit humans and/or animals. Please minimize abbreviations, technical terms, and jargon. When they are necessary, technical terms and abbreviations should be defined.

SECTION E: RATIONALE FOR ANIMAL USE

Assistance from colleagues is very helpful when writing this section. Complete and concise explanations will facilitate the expeditious review of the ASP. Excessive detail should not be confused with "complete" and generally leads to delays in reviewing and approving an ASP.

1) Animal Model: explain why an animal model is necessary for this study as opposed to cell cultures, computer simulations or other non-animal models.

Examples:

This study depends on complex behavioral activities that require a functioning animal with a highly developed nervous system with multiple cell types and a complex multicellular architecture. Cell culture systems are usually composed of a single type of cell that have no multicellular arrangements and can be used to model nervous system function.

The investigation of the effects of anterior hypothalamic lesions on immune responses can only be performed on living organisms with both a well-developed and intact nervous system and an immune system.

Living animal cells are necessary to study the translation of exogenous mRNAs. Oocytes have been used very successfully for these types of studies and are superior to extracts of cultured cells.

2) Appropriateness of the species selected: Describe why the animal model selected is essential for the proposed study as opposed to a lower order vertebrate or an invertebrate. If multiple species are listed for use on the ASP, justify the use of each species separately. If nonhuman primates or other higher-level vertebrates are proposed for use, a thorough discussion and justification of their use must be included.

Example:

This research will examine the genetics of mammary tumors, which occur only in female mammals. Zebrafish strains with mutations in some of the genes of interest have been identified but cannot be used for the study of mammary tumors. In addition, few human or mouse mammary tumors can be grown in tissue culture, making the mouse the ideal organism for generating primary mammary tumors.

3) Estimated Number of animals:

The US Government Principles state that "the animals selected for a procedure should be...the minimum number required to obtain valid results". The goal of Section E3 is to give a good faith account of the Investigator's estimate of the minimum number of animals required to accomplish the research goals.

The Guide for the Care and Use of Laboratory Animals recommends that ACUCs consider "Justification of the species and number of animals proposed. Whenever possible, the number of animals and experimental group sizes should be statistically justified." However, most studies at NIH are either new experimental paradigms or pilot studies, and therefore, applying statistical principles to justify numbers can be difficult due to the lack of knowledge regarding the potential outcomes and variance. Under these circumstances, it is still important to show how the estimated animal numbers were generated and to assert that the sample size reflects the minimum number of animals required. This can be done with a flow chart or table. It is understood that the size of the groups and the number of experiments proposed represents the good faith judgment of the PI and is based on the PI's scientific expertise. Estimates cannot predict the number of animals with precision, so the ASP should state that analysis of the initial experiments will be performed, and the group size adjusted if the results indicate that changes in the size of the experimental groups or the number of experiments will be needed.

Example:
We estimate that we will need to purchase 200 animals for this study because we will be using five (5) animals per group and examining the effects of five (5) compounds (including vehicle), at four (4) doses of drug per compound, with two (2) replications (to assure reproducibility), per determination. Past experience and review of referenced publications (specify) have shown that a group size of 5 provides reasonable assurance of statistical power for this type of study, however if our initial experiments justify a larger or smaller sample size, we will amend our protocol. The total numbers requested will be: 5 x 5 x 4 x 2 = 200.

If the study represents a new animal model, drug or procedure, a statistical analysis may not be appropriate. If the study is a continuation of ongoing work (3-year renewal) or parallels current or previous experiments where the magnitude of the effects and, the degree of variance is known, then a short summary of statistical principles that were applied to arrive at the group size(s) may be appropriate.

When the use of animals is for the harvesting of normal tissues, organs or fluids for *in vitro* use or *in vivo* transfer, briefly cite expected usage levels to provide the quantity of tissues or fluids needed for the study. If no prior experience is available, state an anticipated tissue/fluid harvest per animal with a description of the process.

Example:
In our experience, 10 rats are required to generate enough cells for one experiment. Since we plan to conduct one experiment per week, we need 520 rats per year. This number of animals will enable us to test 10 drugs at the desired dosage.

If rodents are to be used for breeding, then the following categories can be used in justifying and estimating your animal numbers: 1) an estimate of the numbers of progeny intended for experimental use or export, 2) an estimate of the progeny expected, 3) the numbers used for breeding (including founders and initial mates) 4) the numbers of progeny needed for continuation of the experimental line, and 5) the numbers that will be euthanized due to undesirable genotype. An outline, a table, or a flow chart for each strain listed can be very helpful in presenting this information so that the ACUC can assess the appropriateness of requested animal numbers. Excessive detail in this section is frustrating to both the PI and the ACUC and can only lead to delays in ASP approval. Your IC APD, veterinarian, ACUC chairperson and members can be a great help in recommending how to present the breeding scheme.

Example:

We estimate that 100 homozygous mutant animals will be needed to obtain valid results. These animals will be divided into three groups of 15 receiving different doses of the drug, along with 5 untreated controls and parallel groups of wild type animals given the same dosages in two experiments. From our initial founder animals (~3) we will obtain ~10 heterozygous animals along with 10 wild type animals we will use as controls. We will cross heterozygotes to generate ~50 litters of 8 animals each, which should contain 100 homozygous animals if they are born in the expected ratio. [If we find that this breeding scheme does not generate the required number of animals, we will submit an amendment describing the new breeding scheme.] Estimated number of animals: 3 founders + 3 wild type mates (6) to generate 20 F1 progeny, including 10 heterozygotes for breeding. These will be mated and expanded to generate 400 mice of which we estimate that ~100 will be homozygous mutants for our studies. The remainder will be used as controls or for continued breeding or euthanized. Total: 6+20+400 = 426.

For research involving chemical mutagenesis, a statistical justification is appropriate.

Example:

ENU at the dose we have proposed causes mutations in approximately 1 locus out of 1000 loci. Therefore, 1 in every 1000 gametes from a mutagenized male might be expected to carry a mutation in a gene of interest. Based on the frequency of mutations, the following number of animals per year is anticipated:

60 BALB/c males (G0) to be mutagenized in 3 sets of 20. Based on previous experience, we expect that 6/20 mutagenized males will regain fertility after ENU treatment. The fertile males will be mated to 3 C57BL/6J females each to generate 3 litters averaging 8 pups per litter. 6 males x 2 females (one female is used twice) x 8 pups/litter x 3 litters = 192 G1 x 3 ENU treatments/year = 571 G1 progeny, 50% male and 50% female. ~288 G1 females will be discarded. ~ 250 G1 males will be crossed to 3 C57BL/6 females = 750 litters at 8 pups/ litter = 6,000 pups to be screened for dominant mutations. Sperm and tissues from all G1 males will be cryopreserved and archived for sequencing and later mutation retrieval.

60 treated males +36 C57BL/6 females + 576 G1 progeny = 672 mice

250 G1 matings generates 6000 mice for screening

Total Mice = 6672

If the ASP calls for post-harvest use of embryos or fetal tissues, only the dams need be counted, rather than the number of embryos/fetuses. Only if the fetuses are individually used for data collection, such as a fetal surgery experiment, would the fetuses be separately accounted for.

SECTION F: EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES:

The ASP must clearly present the animal procedures. Include enough information in the description of the procedures to enable the ACUC members to understand each procedure and generally how the animals will progress through the individual procedures that are listed. The use of flow charts and tables for more complicated experimental paradigms is often an effective way to satisfy this standard. This part of the form is divided into sections asking for information about different classes of procedures. The description of procedures on the form defines the level of detail desired. You should not provide any more details than is specified on the ASP form unless directed to do so by the IC APD or the ACUC. Include anticipated effects on the animals or personnel coming in contact with the animals. Provide specific details for each procedure that can affect the pain or distress potential for the animals.

Methods of Restraint: All restraint, other than that used for routine procedures, should be described. If prolonged restraint is required, describe: 1) the purpose of the restraint and its

duration, 2) where appropriate, the adaptation training and schedule if compatible with the research objectives of the restraint, 3) frequency of monitoring while in the restraint device, 4) criteria for removal from the device and/or the study if adaptation is not achieved, and 5) consideration of alternatives. Note that alternative restraint methods might include chemical restraint, and if used as such, should list the name of the drug(s) and dose(s) in section I (not in section F).

Injections, Inoculations or Instillations: the use of non-pharmaceutical grade compounds needs to be identified and managed in accordance with the ARAC Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals.

Anesthetic Regimens: Describe in ASP SECTION I.

Hazardous Agents: Describe in SECTION K; include anticipated effects on animals and personnel coming in contact with the animals in SECTION M.

Non-Survival Surgical Procedures**:** non-survival surgery is described in SECTION F. Provide details of survival surgical procedures (both major and minor) in SECTION G.

Special Concerns: Describe in SECTION M.

Behavior Tests: Describe how each test is conducted, how the test contributes to the aims of the study, whether the test is conducted once or multiple times within the same session, the interval between tests or sessions, whether the test or session will be grouped or conducted in parallel with other tests which may involve pain and/or distress, and the test endpoints.

If the behavior test involves pain and/or distress, also describe: the nature and magnitude of the stimulus invoking the pain and/or distress and its control, how much the pain and/or distress the test will cause the animal to experience, measures implemented to mitigate or end the pain and/or distress, and the length of time the animal will be subjected to the painful and/or distressful portion of the test.

Potentially Painful or Distressful Effects: If the animals are a non-regulated species (i.e., mice, rats, birds, fish, etc.) and are expected to experience pain or distress without alleviation with an appropriate anesthetic, analgesic or tranquilizer, then a description of the procedure(s) producing pain and/or distress, and a scientific justification why pain and/or distress cannot be relieved must be provided in this section.

If the animals used are a USDA regulated species (i.e., rabbit, guinea pig, dog, cat, monkey, etc.) then this information needs to be provided both here and on the Column E Form for Regulated Species.

Endpoint Criteria: Provide an experimental endpoint such as a timeline that should be met or medical state that should be achieved (e.g., a 1 cm tumor) that will determine the time for euthanasia or withdrawal from the study. In studies where adverse outcomes or complications might be expected, then humane endpoints should also be described. For example, the pain experienced by an arthritic mouse mutant may be alleviated with analgesics, but lack of proper mobilization may ultimately cause body weight loss and a need for euthanasia prior to reaching the experimental endpoint. In this case, setting a body weight loss standard of 15-20% would constitute a humane endpoint. For all studies, death as an experimental endpoint should be

minimized and must be scientifically justified. Please refer to the ARAC Guidelines for Endpoints in Animal Study Proposals for further details on setting appropriate endpoints for Column D & E studies.

SECTION G: SURVIVAL SURGERY

All Survival Surgery, major and minor, is to be described in this section. In preparing an ASP that includes Survival Surgery, consultation with the IC APD and the facility veterinarian to ensure that the proper techniques are described, and that the facility can support the proposed surgery will facilitate ASP approval.

1) Surgical Procedures and Aseptic Methods: Sterile instruments and aseptic technique are required for ALL species (rodents, rabbits, dogs, etc.). Survival surgery on rodents should be performed in accordance with the ARAC Guidelines for Survival Rodent Surgery.

Describe surgical preparations including pre-operative medications and/or hair clipping and skin disinfection procedures. Describe intraoperative support procedures for the animal, i.e., methods for maintaining body temperature, and methods for assessing depth of anesthesia (heart rate, respiration rate, etc.). Describe methods of instrument sterilization for the initial surgery and (for rodents) between surgeries, i.e., cold sterilant, hot beads, etc. Avoid excessive detail such as the brand name of the instruments, the number of sutures and surgical jargon. Consult with your IC APD and ACUC chairperson about the level of detail required.

2) Surgeon's Qualifications: Provide the names of all individuals performing animal surgery and describe their qualifications to perform the specific procedures listed in terms of related training and experience.

3) Location: Specify the Building and Room number where survival surgeries will be performed. Specialized surgical facilities are required for major operative procedures proposed in rabbits and higher species such as cats, dogs, and primates. Surgery on rodents must be performed in suitably prepared areas in accordance with the ARAC Guidelines for Survival Rodent Surgery.

4) Post-Operative Care: Describe supportive therapy that is required (e.g., supplemental heat source, I.V. fluids, etc.) and state the observations the designated person will use to evaluate the animal's health status in the immediate post-operative period until the animal has recovered from anesthesia. In addition, describe longer term post-operative care needs such as analgesia, suture/staple removal, catheter flushing, etc., that accompanies the surgical procedure(s).

5) Prior Surgery/Multiple Survival Surgeries: If none are proposed fill in "None". If more than one operative procedure is to be performed on a single animal, the need for multiple procedures usually constitutes parts of a single experimental paradigm which must be scientifically justified in this section.

SECTION H: PAIN AND DISTRESS CATEGORY

Pain and Distress: The U.S. Department of Agriculture (USDA) requires an annual report of the number of regulated animals (all mammals EXCEPT rats (of the genus *Rattus)* and mice (of the genus *Mus)* bred for research, and birds) used in research exposed to one of three categories of pain or distress: C (minimal, transient or no pain or distress), D (pain or distress relieved by - anesthetics, analgesics, sedatives or tranquilizers), or E (unrelieved pain or distress). (For examples, see ATTACHMENT 1). ALL NIH ASPs must give the numbers of ALL animals in these three categories. The animal numbers displayed in Section H should match the total numbers in Sections B and E3. If multiple species are used, list each species

separately. Contact your IC APD or an ACUC member for guidance in classifying procedures by the expected levels of pain or distress produced by the procedures.

For ASPs describing animal research categorized as Column D or E, a literature search of at least two different databases must be performed to identify any alternative procedures that are less painful. The search is described in a brief narrative in this section. The majority of NIH researchers meet this requirement by searching databases such as MEDLINE, AGRICOLA or ALTWEB. The written narrative of the database search should contain the databases searched, the date of the search, the years covered by the search, and the keywords and/or search strategy used. The results of the search should be BRIEFLY summarized. Your IC APD or the NIH library staff can provide information on appropriate key words and databases to use for your area of research.

In some highly specialized fields of study: conference proceedings, subject-expert consultants, etc., may provide more relevant and current information. If this method is used, sufficient documentation must be provided to demonstrate the viability of this information in addressing the issue of alternatives.

SECTION I: ANESTHESIA, ANALGESIA, TRANQUILIZATION

Completing this section should involve consultation with the IC APD and the facility veterinarian. The type and dose of anesthetic, analgesic, and tranquilizer or sedative must be appropriate for both the species being used and the type of pain or distress being prevented/relieved. Doses and routes of administration should be clearly appropriate and effective, i.e., commonly accepted or published doses, or through a description of your experience with the agent and dose described which demonstrates its effectiveness.

The frequency &/or indications for drug administration should be provided, e.g., every 12 hours, as needed, etc. If agents are to be given "as needed", a brief description of the indications for its administration should be provided, e.g., "at the first indication of discomfort as evidenced by lethargy, anorexia, hunched posture, eye squinting, or vocalization".

If anesthetics, analgesics or tranquilizers must be withheld when a procedure will cause more than slight or momentary pain or distress, then it must be scientifically justified, see Section F.

The compounds used must also be pharmaceutical grade or justified IAW the ARAC Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals.

SECTION J: METHOD OF EUTHANASIA

A description of euthanasia for ALL animals on the ASP must be clearly described in this section. For most ASPs, the methods described in the ARAC Guidelines for the Euthanasia of Rodent Fetuses and Neonates, Guidelines for Euthanasia of Rodents Using Carbon Dioxide and Guidelines for Use of Zebrafish in the NIH Intramural Research Program can be cited. Methods which are not consistent with the recommendations in the AVMA Guidelines for the Euthanasia of Animals: 2013 **Edition** must be scientifically justified in the ASP.

Animal carcasses which have NOT been contaminated with hazardous agents are to be disposed of as Medical Pathological Waste in accordance with NIH Office of Research Facilities Development and Operations, Division of Environmental Protection guidelines. Each animal facility has facility-specific methods of disposal, which can be obtained from the IC APD of the facility veterinarian.

The Office of Research Facilities Development and Operations, Division of Environmental Protection personnel (301-496-3537), the DOHS representative on the ACUC and the facility veterinarian should be

consulted on proper disposal methods for carcasses contaminated with hazardous agents. The proper disposal of contaminated carcasses should be described in Section K, NOT HERE.

SECTION K: HAZARDOUS AGENTS

Your ACUC Chairperson or Coordinator can refer you to your ACUC assigned DOHS Health and Safety Specialist for assistance in describing the use of Hazardous Agents in animal research. The DOHS representative will assist you in obtaining all of the appropriate safety documents specified in this section (e.g., radiation safety protocols, Recombinant DNA documents, etc.), which should be submitted as attachments. It is imperative to contact the DOHS representative early in the ASP development phase as the use of hazardous agents requires the signature of the DOHS Safety representative(s) under Section O before an ASP can be approved. The description of the agents on the form defines the level of detail required for this section. The disposal of carcasses contaminated with Hazardous Agents should be described here as well.

Ionizing Radiation: Identify radioactive isotopes and other ionizing radiation sources used *in vivo* and their activity. A Division of Radiation Safety (DRS) Health Physicist signature is required. Contact DRS at: 301-496-5774 for additional information.

Biological Agents with Pathogenic Potential: List viruses, bacteria, and any blood or body fluids potentially infectious to humans or any human tissue, blood, cells, etc. to be used. A Pathogen Registration Document (PRD) must be filed with the DOHS for the use of these biological agents. Identify the PRD number.

Recombinant DNA: Identify any nonexempt Recombinant DNA used in vivo e.g. constructs used for development of transgenic or knock-out animal models. A recombinant DNA registration document must be filed with and approved by the NIH Institutional Biosafety Committee prior to initiation of the study; unless the recombinant rDNA qualifies for registration simultaneous with initiation, e.g. ABSL-1 constructs. Verify RDNA requirements with the NIH IBC.

Hazardous Chemicals or Drugs: List any hazardous chemicals or drugs (carcinogens, mutagens, formaldehyde, inhalant anesthetics, etc.).

SECTION L: BIOLOGICAL MATERIAL/ANIMAL PRODUCTS FOR USE IN ANIMALS

Biological material and animal products such as cell lines, tissues, and tumors that are introduced into research animals can harbor animal pathogens (e.g., ectromelia, lymphocytic choriomeningitis and mouse hepatitis) which can then infect NIH animal colonies. ACUC approval is required prior to introducing any rodent, rodent product or biological material that originates from sources other than those approved by the Division of Veterinary Resources. (See the NIH Policy Manual 3043-1, Introduction of Rodents and Rodent Products.)

Principal Investigators are responsible for ensuring that the biologic materials used in their study will not endanger the health of the live animals used in their study or other animals housed in the animal facility. To avoid delays in approval, the testing of Biologic Material/Animal Products is ideally done prior to ASP submission. Your IC APD can provide you with information about the kinds of tests required.

In this section, the PI describes the materials, the tests performed, and certifies that the material can be used safely in the relevant animal facilities. The documentation of the testing should be submitted as an attachment.

SECTION M: SPECIAL CONCERNS OR REQUIREMENTS

List any items or procedures that may require special care or attention by either the PI or the animal facility during the performance of the study. Include procedures that may adversely affect the animals, how those effects will be detected, and the actions that will be taken to support the animals and to minimize pain or distress.

Information regarding and identification of animals that may require special care due to surgical alterations (e.g., splenectomy, adrenalectomy, etc.) or genetic manipulations should be recorded.

List any unusual requirements that the animal facility management may need to consider supporting the study, including importation, specialized housing (e.g., sterile cages for immunocompromised animals), lighting, feed, water or a need for other than routine veterinary care. Also include the use and storage of specialized pieces of equipment and special off-hour or weekend/holiday requirements.

If nonhuman primates must be exempted from all or part the facility enrichment plan or if dogs must be exempted from the facility exercise plan, this must be scientifically justified in this section.

SECTION N: PRINCIPAL INVESTIGATOR CERTIFICATIONS

All ASP forms must be signed by the Principal Investigator. The signature of the Principal Investigator in this section certifies the following:

1) That the PI has completed the OACU "Guidelines for Principal Investigator" course, which is offered as web based training. To access the web based training, you can go to the OACU training website at: https://oacu.oir.nih.gov/ . For additional information contact your IC ACUC Coordinator or the OACU Associate Director for Training (301-496-5424).

2) That the PI certifies that the proposed research is not duplicative of previous research.

3) That the PI and all personnel listed in Section A are enrolled in the OMS Animal Exposure Program (AEP) or equivalent. If information or assistance is needed for enrolling in the Program, contact your IC ACUC Coordinator or OMS at 301-496-4411. (http://www.ors.od.nih.gov/sr/dohs/OccupationalMedical/Pages/oms_main.aspx)

4) That all personnel listed in Section A have completed the OACU "Guidelines for Animal Users" course and been trained in all procedures described in the ASP. The signature also certifies that the PI IS RESPONSIBLE for ALL of the animal-related activities of these personnel. The "Guidelines for Animal Users" course is offered as web-based training. To access the web-based training go to the OACU training website at: https://oacu.oir.nih.gov/. For additional information, contact your IC ACUC Coordinator or the OACU Associate Director for Training (301-496-5424).

5) That the PI has "considered alternatives to procedures that may cause more than momentary or slight pain and distress and has provided a written narrative description of the methods and sources ... used to determine that alternatives were not available" as specified by the Animal Welfare Act Regulations. This narrative appears in Section H of the ASP form.

6) That the PI will report all significant changes in writing, to the ACUC for review and approval prior to the initiation of the study change. The ARAC Guideline Regarding Significant Changes to Animal Study Proposals defines significant changes as those that have the potential to impact substantially and directly on the health and wellbeing of the experimental animals. For minor ASP changes, refer to your IC ACUC Chair or APD for proper reporting procedures.

SECTION O: CONCURRENCES

It is the responsibility of the Principal Investigator to obtain the signature of the Laboratory/Branch Chief. Proposals originating from a Laboratory/Branch Chief require the concurring signature of the Scientific Director. This signature certifies that the research program of the PI has been reviewed and approved for scientific merit and that the resources to conduct this research are available.

The signature of the pertinent DOHS Health and Safety Specialist, Facility Veterinarian, etc., must also be obtained prior to ASP approval.

The Facility Staff only certifies that it has reviewed the ASP and has the infrastructure and veterinary care resources/information to support the ASP. Approval is not withheld or delayed for other reasons. If the Facility Staff has questions outside of the areas of infrastructure and veterinary care, those questions must be presented in writing to the Chair of the ACUC responsible for the ASP.

SECTION P: FINAL APPROVAL:

The Chairperson of the IC ACUC has authority for final approval of the ASP.

Revised and approved by ARAC as a "useful document": 04/09/2003, 08/10/2005, 07/12/2006
Approved as an ARAC Guideline: 12/18/2007
Revised: 07/11/2012; 04/09/2014; 02/22/2017; 12/09/2020

Instructions for the "Column E: Explanation Form for Regulated Species"

If your study involves procedures that are categorized as "Column E" under section H, animals that will suffer from "unrelieved pain or distress," and they are a regulated species (i.e., rabbit, guinea pig, dog, cat, monkey, etc.), then this form must also be filled out. This information is then forwarded to USDA as part of NIH's annual reporting requirements.

Procedures producing pain and/or distress, including reason(s) for species selected.
This should be a concise explanation pulling in parts of the scientific basis of the study that support the use of this model. This should match the information provided in Section F.

Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. Provide summary of supportive care measures (if applicable).

Rather than referring the reader to Section H of the protocol, this description should cite past research experience and possibly key papers that support the justification. This is also a good place to briefly discuss measures that are being taken to minimize the level of pain and distress the animals will feel such as medical care procedures, increased monitoring during critical phases, and/or specific humane endpoints that are proposed in Section F.

Instructions for the "Emergency Animal Treatment & Care Form"

- The NIH intramural ICs will use the standard format provided which will serve as the minimum information to be gathered.
- Additional requested information may be added to this form at the IC's discretion.
- Changes in the format will be considered for approval by the OACU.
- Fill out one form for the ASP and submit it along with the completed ASP for ACUC review.
- Multiple forms may be used if care is different for multiple species listed on the ASP or if care is different for individual experimental groups.
- All information listed on this form should be the same as listed in the ASP *without contradiction* regarding animal care issues or endpoints.
- Animal housing location – this is best established prior to completion of the form as the location may dictate the type and level of care that can be provided.
- List of procedures – this should emphasize procedures that may result in serious animal health complications.
- Points of contact (POC) – this does not have to be the PI but should be the person working directly with the animals who has an intimate knowledge of their experimental status and overall health.
- Potential or expected complications – this should be directly tied to the "list of procedures" and therefore if there are multiple procedures listed, there will likely be multiple complications listed.
- Circumstances requiring contact - this should be directly tied to the "list of procedures" and therefore if there are multiple procedures listed, there will likely be multiple circumstances listed.
- Treatment – if the treatment cannot be prescribed by the veterinarian, then please be diligent in listing restrictions and specific treatments for all listed complications.
- Euthanasia – if euthanasia is not at veterinary discretion, then please be diligent in listing restrictions and specific criteria for all listed complications. If the POC must be notified prior to euthanasia, then please ensure accurate contact information and POC availability at critical stages of the experiment.

Instructions for Documenting "Training & Experience"

- The NIH intramural ICs will use the following guidelines which will serve as the minimum information to be gathered.
- Additional requested information may be added to this form at the IC's discretion.
- Changes in the format will be considered for approval by OACU.
- ICs may compile the Training and Experience data as a database in the ACUC Coordinator's office and/or may request that investigators complete a separate form for the PI and each animal user listed under Section B of the ASP form and submit the forms along with the completed ASP.
- The forms are subject to ACUC review or by an agent of the Committee.

General Information

PI (Principal Investigator) & AU (Animal User) course completion dates. Training record dates can be obtained by contacting your IC ACUC Coordinator or from the OACU Student Training History Website: https://federation.nih.gov/oacu/.

Training & Experience (T&E)

- This should only address the Animal User's T&E for the procedures listed in this ASP that they will actually be performing and should address both experimental procedures, i.e., injections, blood withdrawals, etc., as well as surgical procedures (unless T&E is recorded centrally per individual instead of per ASP).
- Short, bulleted statements are sufficient.
- Name of PI &/or designee - the person responsible for supervision and training does not have to be the PI but should be the person that has the particular skills and daily interaction to provide training and oversight.

Nonhuman Primate Procedures

- This section is completed only if the ASP involves the use of nonhuman primates.
- "Awake" procedures are any activities and/or manipulations that would place an individual at reasonable risk of contact with an awake monkey or its feces or body fluids. Examples of awake procedures would include placing food in a cage or feedbox, using a squeeze back mechanism, jumping a monkey to another cage or transport device, pole and collar work, placing in a chair or other restraint device or behavior recording within a two foot radius (definition per PM 3044-2).

Assurances

- If a paper form is used, both the Animal User and the PI must sign the form prior to submission.
- If a centralized T&E data collection methodology is used, the Animal User must verify that he/she has read all ASPs to which they are assigned, and the PI (or designee) must verify that the Animal Users procedural proficiency has been assessed.

ATTACHMENT 1: Guidelines for Pain/Distress Classification

Definitions:

***Pain** is an unpleasant sensory or emotional experience associated with actual or potential tissue damage or described in terms of such damage.[1]

***Distress** is an aversive state in which an animal fails to cope or adjust to various stressors with which it is presented.[2]

([1]Recognition and Alleviation of Pain in Laboratory Animals, National Research Council, 2009)

([2]Guide for the Care and Use of Laboratory Animals, National Research Council, 2011)

Column C - Minimal, transient, or no pain or distress

These procedures are considered to produce minimal, transient, or no pain or distress when performed by competent individuals using recognized methods.

1. Administration of:
    a. Fluid and electrolyte therapy
    b. Immunizations
    d. Oral medications
    e. Anesthetics, analgesics and tranquilizers
2. Peripheral catheterization
3. Blood collection (except intracardiac, and periorbital in some species, see below)
4. Gastric gavage
5. Certain procedures performed in the normal practice of veterinary medicine and those involving the diagnosis and treatment of disease (e.g., injections, palpations, skin scraping, radiography).
6. Euthanasia as performed in accordance with recommendations of the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.
7. Intracerebral inoculations in neonatal rodents. In many neonatal rodents intracerebral inoculations can be performed by trained personnel prior to cranial ossification, producing only transient pain or distress.

Column D - Pain or distress relieved by appropriate measures

Examples of procedures that may produce pain or distress, but when performed using appropriate and adequate anesthetics, analgesics, sedatives or tranquilizers and followed with appropriate measures to alleviate pain or distress are as follows:

1. All surgery, (major, minor, non-survival) including biopsy, gonadectomy, and neurophysiological manipulations or preparations such as implantation of electrodes and recording devices, and alterations to nerve or muscle fibers.
2. Fracturing bones.
3. Intra-cardiac blood collection.
4. Periorbital collection of blood from any species except mice and hamsters. Note: Periorbital collection from unanesthetized animals that do not possess a true orbital sinus, as do mice and hamsters, is discouraged.

Column E - Unrelieved pain or distress

Procedures, including those listed above for Column D, which are performed without appropriate and adequate anesthesia, analgesia, or tranquilizers; or which are not followed with appropriate measures to alleviate pain or distress; or which are not amenable to relief by therapeutic measures, must be listed in Column E.